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(71) Applicant *(for all designated States except US)*: **MILTENYI BIOTEC GMBH** [DE/DE]; Friedrich Ebert Str. 68, 51429 Bergisch Gladbach (DE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **PIECHACZEK, Christoph** [DE/DE]; Briloner Strasse 11, 51109 Köln (DE). **BETHKE, Ulf** [DE/DE]; Hauptstrasse 305, 51465 Bergisch Gladbach (DE). **WIDERA, Darius** [DE/DE]; Luxemburger Strasse 46, 50674 Köln (DE). **SCHMITZ, Jürgen** [DE/DE]; Josef-Thüner-Strasse 34, 50126 Bergheim (DE).

(74) Agent: **STERNAGEL, FLEISCHER, GODEMEYER & PARTNER**; An den Gärten 7, 51491 Overath (DE).

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(54) Title: A METHOD FOR GENERATING HUMAN NERVOUS SYSTEM CELLS, TISSUES, OR NEURAL STEM CELL PROGENITORS FROM HAEMATOPOIETIC STEM CELLS

(57) Abstract: This invention relates generally to a method for the generation of human cells and /or tissues of the nervous system or neural stem cell progenitors from human hematopoietic stem cell progenitors and to the use of human hematopoietic stem cell progenitors for the manufacture of a medicament for preventive and/or therapeutic treatment of injuries of the central nervous system, peripheral nervous system or for preventive and/or therapeutic treatment of neurodegenerative diseases.

A METHOD FOR GENERATING HUMAN NERVOUS SYSTEM CELLS, TISSUES, OR NEURAL STEM CELL PROGENITORS FROM HAEMATOPOIETIC STEM CELLS

Technical Field

This invention relates generally to a method for the generation of human cells and/or tissues of the nervous system or neural stem cell progenitors.

Background of the invention

Current treatments for acute neural injury is primarily medical with the use of high dose steroids (Barken et al. in N. England J. Med. (1990) 322: 1405-1411), and supportive through aggressive nursing care and rigorous rehabilitation. Surgical interventions are primarily aimed at spinal cord stabilisation (Lee et al. in Neurosurgery Quarterly (1999) 9: 138-153). Recently there has been an effort to examine the role of aggressive decompressive surgery in spinal cord injury (Fehlings and Tator in J. Neurosurg. (1999) 91: 1 – 11 and Tator et al. in J. Neurosurg. (1999) 91: 12 – 18. To date there is no therapeutic intervention to significally restore functions after spinal cord injury.

Medical advancements have significantly increased the survival of spinal cord injury patients, restoration of functions, however, has not yet been approved. Neural transplantation has been studied the past decade in animal models as a repair strategy for spinal cord injury as well as for neuro-degenerative diseases like Parkinson's and Huntington's disease. Although no neural transplantation has yet to reach the point of clinical application reconstructive strategies offer a solution for the treatment of neural injury of dysfunction. One of the major problems is the choice of the transplanted cell type. Tissue sources for transplantation have involved peripheral nerve grafts, dorsal root ganglia, Schwann cells, adrenal tissue and fetal spinal cord tissue derived from human sources.

Neural transplantation for spinal cord injury have been reported in some clinical trials. A trial of transplanting fetal neocortex into 41 patients with chronical spinal cord injury was performed in Russia about a decade ago (Reier et al. J. Neurotrauma (1994) 11: 396-377). No long-term follow-up or evidence of graft survival is available but the authors reported an improvement in sensory function of a number of dermatomes in some patients. A team reported the use of human embryonic spinal cord tissue to obliterate a post-traumatic syrinx (Falci et al. in J. Neurotrauma (1997) 14: 875-884). The authors reported a seven-month follow-up with persistent obliteration of a 6-cm cavity and good visualisation of the graft on MR imaging.

Different experiments were done by transplanting nerval tissue obtained from human fetusses for treatment of diseases like Parkinson's disease (Price et al. in Biol. Psychiatry (1995) 38 (8) 498-505; Lopez-Lozano et al. in Transplant Proc. (1995) 27 (1): 1395-1400; and Widner et al. in N. England J. Med. (1992) 327 (22): 1556 – 1563, Huntington's disease (Bachoud-Levi et al. in Exp. Neurol. (2000) 161 (1) 194-202 and in Lancet (2000) 356 (9246): 1975 – 1979) or diseases of the retina (Humayun et al. in Invest Ophtalmol. Vis. Sci. (2000) 41 (10) 3100 – 3106). Porcine fetal neural cells were used for xenotransplantion to treat both, Parkinson's and Huntington's disease (Fink et al. in Cell Transplant (2000) 9 (2): 273 – 278).

Autologous neural cells obtained by perfusion of the patient´s adrenal medulla were used to treat Parkinson's disease to circumvent one of the major problems of non-autologous cell transplantation, the host versus graft disease (Lopez-Lozano et al. in Transplant Proc. (1990) 22 (5): 2243 – 2246). A combination of nerval tissue obtained from human fetusses and autologous cells of the adrenal medulla was also used to treat Parkinson's disease (Lopez-Lozano et al. in J. Neurosurg. (1999) 90 (5) 875-882).

Recently research has been focussed on stem cells in order to find a therapy for disorders involving destruction and degeneration of tissues, such as spinal cord

injuries, Parkinson's disease and other neuro-degenerative diseases, which are accompanied by insufficient or non-existing cell renewal.

In the developing embryo cells derived from the embryonic germ layers become committed to a particular differentiation pathway acquiring specific morphologies and patterns of gene expression which permit them to fulfil their designated roles. But the capacity for growth and differentiation is not entirely lost in adults. Even differentiated organs and tissues can sometimes be generated from stem cells that reside in the tissues.

The most common definition of stem cells refers to their ability for self renewal. Stem cells are defined as cells having the ability, both to divide and give rise to more stem cells. They can also divide to give rise to differentiated cells which are *often differentiating into multiple pathways. The archetype of stem cells is the* embryonic stem cells. Embryonic stem cells usually derive from early stage embryos and as totipotent cells they can give rise to all of the adult tissues.

Tissue specific stem cells are generally believed to have a developmental commitment to a class of cells and are usually localised to specific sites in the corresponding organ. As an example hematopoietic stem cells are found in high numbers in bone marrow. The high turnover of mammalian. blood cells requires a supply of hematopoietic stem cells which are able to give rise to other blood cell lineages These blood cell lineages include monocytes and lymphocytes. The immediate progeny of hematopoietic stem cells is believed to be "progenitor" cells, which are capable of giving rise to various cell types within one or more lineages, i.e. the erythroid, myeloid and lymphoid lineages. The stem cell and progenitor cell populations constitute only a small percentage of the total number of cells in bone marrow, fetal liver, etc.. These populations are of immense interest because of their ability to repopulate the hematopoietic system. The longevity of stem cells and the dissemination of stem cell progeny are desirable characteristics. There is a significant commercial interest in these methods because stem cells have a number of clinical uses. Progenitor cell transplantation is currently used in conjunction with chemotherapy and radiation for the treatment of leukemia, breast

cancer and other tumours. Frequently autologous transplants are used to avoid the danger of graft rejection but there is an increased risk of disease reappearance due to the presence of tumour cells in the engrafting cell population. Transplantation of a more purified source of progenitors cells is therefore preferable.

The ability to culture somatic stem cells holds great promise for therapy in diseases involving destruction and degeneration of tissues, such as spinal cord injuries, Parkinson's disease and other neuro-degenerative diseases. For these reasons the isolation, characterisation and manipulation of stem cell progenitors is of great interest.

Proteins and other cell surface markers found on hematopoietic stem cell and progenitor cell populations are used for preparing reagents for the separation and isolations of these populations, and in the further characterisation of these important cells.

Hematopoietic stem cells are characterised by the expression of hematopoietic cell surface antigens like CD34, CD133 or CD117. CD34+ cells have been isolated from peripheral blood and bone marrow for the first time in 1989 and have been described as a long term repopulating cells in vivo and in vitro. Under basis of this data methods have been developed for the isolation of CD34 antigen expressing cells and for the use of this cells for therapeutic treatment after myeloablative chemotherapy. In 1998 the CD133 antigen was described and used as a marker for long term repopulating cells in NOD-SCID mice and in fetal sheep transplantation model. The CD133 antigen belongs to a new glycoprotein family characterised by five transmembrane domains. CD133+ cells are also positive for the expression of CD34 and CD45 which are identification markers for blood cells, and are also partially positive for the expression of CD117 and CD38.

US Patent No. 5,843,633 describes the monoclonal antibody called anti-AC133 (the antigen AC133 was renamed as CD133) which binds to a surface marker glycoprotein on hematopoietic stem and progenitor cells. The CD133 antigen is a

5-transmembrane cell surface antigen with a molecular weight of 117 kDa. The expression of this antigen is highly tissue-specific and has been detected on a subset of hematopoietic progenitor cells derived from human bone marrow, fetal bone marrow and liver, cord blood and adult peripheral blood. In the hematopoietic system CD133-expression is restricted to the subset of CD34+^{bright} stem and progenitor cells in human fetal liver, bone marrow, cord blood and peripheral blood and additionally to a small portion of CD34– cells. The CD34+ CD 133+ cell population including colony forming unit granulocyte macrophage (CFU-GM) as well as CD34+ CD38- cells was shown to be the only one capable of repopulating NOD/SCID mice (De Wynter et al. in StemCells (1998) 16:387-396). CD133 has been shown to be expressed on endothelial precursor cells (Gehling et al. in Blood (2000) 95: 3106-3112, and Peichev et al. in Blood (2000) 95:952-958).

The CD133 antigen has also been shown to be expressed on fetal neural stem cells (Uchida et al. in PNAS (2000) 97:14720 – 14725; WO 00/47762). In all cases which have been examined the CD133 antigen is found concentrated in microvilli and other plasma membrane protrusions (Corbeil et al. in J. Biol. Chem. (2000) 275: 5512-5520). The function of the CD133 molecule is unknown and no natural ligand has yet been found.

US Pat. No. 5,061,620 describes a substantially homogeneous human hematopoietic stem cell composition and the manner of obtaining such composition. Stromal cell-associated hematopoiesis is described by Paul et at. (1991) Blood 77: 1723-1733. The phenotype of stem cells with rhodamine staining is discussed in Spangrude and Johnson (1990) P.N.A.S. 87:7433 – 7437. Cell surface antigen expression in hematopoesis is discussed in Strauss et al. (1983) Blood 61: 1222 – 1231 and Sieff et al. (1982) Blood 60: 703 – 713. Descriptions of pluripotential hematopoietic cells are found in McNiece et al. (1989) Blood 74: 609 – 612 and Moore et al. (1979) Blood Cells 5:297-311. Characterisation of a human hematopoietic progenitor cell capable of forming blast cell-containing colonies in vitro is found in Gordon et al. (1987) J. Cell. Physiol. 130:150-156 and Brandt et al. (1988) J. Clin. Invest. 82:1017-1027. The use of progenitor cells in transplantation

is discussed in To et al. in Progenitor Threshold in Transplantation (ISBN 1-88085417-1) pp. 15-20.

The use of high gradient magnetic separation for the isolation of human hematopoietic progenitor cells is described in Thomas and Landsdorp (1992) in Advances in Bone Marrow Purging pp. 537 – 544; and Kato and Radbruch (1993) Cytometry 14:384-392. Other methods of magnetic selection for human hematopoietic progenitor cells are described in Bigas et al. (1992) in Advances in Bone Marrow Purging pp. 545 – 551; Oku et al. (1992) in Advances in Bone Marrow Purging pp. 553 – 560; and Hardwick et al. (1992) in Advances in Bone Marrow Purging pp. 583 – 589. High gradient magnetic cells sorting is described in Miltenyi et al. (1990) Cytometry 11:231 – 238. Molday, US Pat. No. 4,452,773 describes the preparation of magnetic iron-dextran microspheres and provides a summary describing the various means of preparation of particles suitable for attachment to biological materials.

Recently, attempts have been made to use hematopoietic stem cell for the generation of non-hematopoietic cells and tissues. In WO 01/71016 a method is disclosed for the generation of hepatocytes from murine $Sca1^{+}$, $Lin^{-/lo}$ hematopoietic stem cells isolated from the bone marrow. However, it has not been demonstrated how to generate human hepatocytes or cells of a different type. As a matter of fact murine cells do not express CD133 antigen. Furthermore, by using murine stem cells there is a great risk of transferring microbial, viral and spongiform agents if human beings are to be treated.

Other attempts refer to the generation of cells of the nervous system. Uchida et al. (PNAS (2000) vol. 97 No. 26, pp. 14720-14725; and WO 00/47762) disclose that in fetal tissue CD133 antigen was expressed on the cell surface of neural stem cell progenitors. Also methods are disclosed for identifying, isolating and enriching human central nervous system cells and neural stem cells which can initiate neurospheres and progenitor cells. However, these cells are of non-hematopoietic origin since hematopoietic markers such as CD45 or glycophorin A have been shown not to be expressed in these cells. Cells of the nervous system have

ectodermal origin. Furthermore, for this method the cells have been prepared from fetal brain. As stated in this application the cells can be derived from late embryo, juvenile or adult mammalian central nervous system tissue.

The disadvantage of the state of art is that the methods used do not allow collecting and isolating these cells from the donor without a major damage. The use of fetusses, which are destroyed when used as a source for receiving the target cells is also not be favoured. Furthermore, the generation of human neural cells from other cells than neural cells or neural stem cells has not yet been shown.

The object of the present invention was to provide a method for the generation of human cells and/or tissues of the nervous system and/or neural stem cell progenitors wherein the cells can be collected from a donor without a major damage. It is an object of the invention to provide a method without having to use and to destroy fetusses. A method was to be provided, wherein the cells can be collected without a major damage for the donor.

It is also an object of the invention to provide compositions and medicaments which can be used in xenogenic, allogeneic or autologous transplantation, in order to restore neural tissue. The object of this invention is therefore to provide compositions containing precursor cells with the potential to restore neural tissue.

The technical problem is solved by providing a method for the generation of human cells and/or tissues of the nervous system or neural stem cell progenitors from human hematopoietic stem cell progenitors.

The present invention provides methods for the generation of human cells and/or tissues of the nervous system or neural stem cell progenitors from human hematopoietic stem cell progenitors. According to the method of the present invention the hematopoietic stem cell progenitors are induced to differentiate into cells of the nervous system which may comprise one or more cells selected from

the group neurons, glial cells, Schwann cells, astrocytes, oligodendrocytes. The differentiation may be in vitro or in vivo.

The hematopoietic stem cell progenitors may preferably isolated from umbilical cord, peripheral blood or bone marrow. In contrast to the above mentioned prior art the present invention exclusively makes use of non-fetal hematopoietic stem cells. By using umbilical cord, peripheral blood or bone marrow as a source of the hematopoietic stem cell progenitors the collection of said cells will be of no harm for the donor. According to the method of the present invention the use of embryonic stem cells or other cells from the developing embryo can be avoided. Therefore, no fetusses have to be destroyed.

Furthermore, it has been shown for the first time that cells of the nervous system may be generated by using hematopoietic stem cell progenitors and inducing the differentiation of these cells to neural cells.

It shall be noted that the mixed population of cells containing hemotopoietic stem cell progenitors, which are isolated from umbilical cord, peripheral blood or bone marrow normally do not contain cells of the nervous system or their precursors. The cells of the nervous system or the neural stem cell progenitors which will be generated by the method according to the present invention are derived from cell of the hematopoietic system, namely hematopoietic stem cell progenitors.

A population of cells having the phenotype of human hematopoietic stem cell progenitors have "multipotent" developmental potential and they can give rise to cells and/or tissues of the nervous system as was found by the inventors of the present invention. The term "multipotent" hematopoietic stem cell progenitors refers to cells that are capable of self-generation during propagation, and which have the capacity in vitro or in vivo to differentiate into lineage committed cells that further proliferate and terminally differentiate into cells of the nervous system. Such multipotent hematopoietic stem cell progenitors may differentiate into cells of the nervous system, including neurons, glial cells, Schwann cells, astrocytes and oligodendrocytes, through in vitro or in vivo induction.

The hematopoietic stem cells can be isolated with low effort and are used for the generation of human cells and/or tissues of the nervous system or neural stem cell progenitors. The generated cells may be used for therapeutic treatment for example in autologous, allogenous or exogenous transplantation. The transplantation of the isolated cells or the transplantation of their progeny cells which were generated by in vitro cultivation allows the substitution of damaged or dead cells in tissues which have limited self-renewal capacities. There are also methods for cultivation provided on the basis of defined media and additives which minimise the risk of transferring microbial, viral and spongiform agents.

In a preferred embodiment the isolated human hematopoietic stem cell progenitors are cultivated and/or propagated in vitro or used for transplantation directly after isolation. A method is provided for the the propagation and maintainance of human hematopoietic stem cell progenitors.

In vitro cultivation of adult hematopoietic stem cell for propagation and differentiation into hematopoietic stem cells and effector cells has extensively been studied and is used as quality control for stem cell preparations. In the literature this assays are called CFU (Colony Forming Unit). Particularly following assays have been provided:

1) CFU-A which can detect multipotent hematopoietic stem cells
2) CFU-C for the detection of granulocyte progenitor, which is not of the type of the earliest stage of hematopoietic stem cells (CFU-GM)
3) CFU-E for the detection of earliest stage erythroid progenitor cells
4) CFU-Eo for the stimulation of colonies of eosinophils
5) CFU-G for the stimulation of colonies of granulocytes
6) CFU-M for the stimulation of colonies of monocytes
7) CFU-GEMM for the generation of colonies of the myeloid blood cell line
8) CFU-MEG for the stimulation of colonies of megakaryocytes.

With all these methods blood cells expressing the markers of the hematopoietic system such as CD45 or erythrocyte markers such as glycophorin A may be generated in vitro. In any case these stem cells fully differentiate during their in vitro cultivation. Until now it has not been shown that cells cultivated in vitro in a serum free medium maintained their capacity or potential as long term repopulating cells.

The following preferred features of the method according to the present invention refer to the hematopoietic stem cell progenitors before the induction of the differentiation. In a preferred embodiment of the method for the generation of human cells and/or tissues of the nervous system a population of cells is used wherein at least about 5 % of the cells present having the hematopoietic stem cell progenitors phenotype, preferably at least about 10 % of the cells present. Preferably the cell population comprises cells expressing the cell surface marker antigen CD45. More preferred the cell population contains at least 90 % of cells expressing the cell surface marker antigen CD45.

The cell population used may also contain at least 10 % of cells expressing the cell surface marker antigen CD133. The cell population may also express CD117 antigen. According to this invention cells expressing one or more of the cell surface antigens CD133, CD117, CD34 will be used. Each single cell of the hematopoietic stem cell progenitors does express one or more of the cell surface marker antigens selected from the group CD45, CD34, CD133 and CD117.

In a preferred embodiment the method of the present invention comprises the steps of:

a) obtaining a mixed population of human cells comprising hematopoietic stem cell progenitors from peripheral blood and/or bone marrow and/or umbilical cord blood,

b) enrichment of the hematopoietic stem cell progenitors by selecting for those cells that are CD34 positive and/or CD117 positive and/or CD 133 positive,

and additionally to the enrichment step or instead of the enrichment step: depletion of those cells that are in a more differentiated state than the hematopoietic stem cell progenitors.

c) induction of the differentiation of the hematopoietic stem cell progenitors into cells of the nervous system or neural stem cell progenitors by placing the cell population comprising enriched hematopoietic stem cell progenitors in an appropriate cell-inducing environment.

In the depletion step, preferably those cells are depleted having cell surface antigens selected from the group CD2, CD3, CD14, CD16, CD19, CD24, CD56, CD66b, Glycophorin A. The advantage of the depletion step is that those cells are removed which are in a more differentiated state than the hematopoietic stem cell progenitors, i.e. especially such cells are removed which are determined in one or more of those characteristics which are defining blood cells. The depletion step is performed by contacting the mixed cell population with one or more reagents that bind to the respective antigen and removing the cells that have bound the reagent, to produce a population enriched for hematopoietic stem cell progenitors. The reagent may preferably be an antibody.

In a preferred embodiment the method is carried out using both the enrichment step and the depletion step. If the method is applied wherein the enrichment step and the depletion step is applied, the depletion step may also be carried out after the induction of the differentiation, for example at any stage of in vitro culture of the cells.

The method according to the present invention provides human cells and/or tissues of the nervous system or neural stem cell progenitors which are generated from human hematopoietic stem cell progenitors, wherein a mixed population of human cells comprising hematopoietic stem cell progenitors is obtained from peripheral blood and/or bone marrow and/or umbilical cord blood; after isolation the hematopoietic stem cell progenitors are enriched by selecting for those cells that are CD34 positive and/or CD117 positive and/or CD133 positive; after

enrichment the differentiation of the hematopoietic stem cell progenitors is induced into cells of the nervous system or neural stem cell progenitors by placing the cell population comprising enriched hematopoietic stem cell progenitors in an appropriate cell-inducing environment.

Preferably the enrichment in is performed by contacting the mixed cell population with one or more reagents that bind to antigens selected from the group: CD34, CD117, CD133; and selecting for cells that have bound the reagent, to produce a population enriched for hematopoietic stem cell progenitors. The reagent may preferably be an antibody.

More preferred, after the enrichment the cell populations are at least about 5 % of the cells present having the hematopoietic stem cell progenitors phenotype, preferably at least about 10 % of the cells present. These cells express are CD45+ indicating their hematopoietic origin. Preferably, after enrichment the population contains at least 90 % of cells expressing CD45.

In a further embodiment the cells are cultivated in a medium supplemented with growth factors which induce the differentiation of human hematopoietic stem cell progenitors into cells of the nervous system or neural stem cell progenitors.

According to the present invention the medium used preferably was supplemented with growth factors which induce the differentiation of human hematopoietic stem cell progenitors into cells of the nervous system or neural stem cell progenitors. Surprisingly it was found that by use of defined media and supplementation of factors specific for the differentiation of cells of the nervous system hematopoietic stem cell progenitors differentiate into a cell population containing one or more cell types selected from the group Schwann cells, astrocytes, glial cells and oligodendrocytes. The differentiation of these cell types is preferably achieved without the use of human or animal serum in order to exclude the risk of an infection by transferring microbial, viral or spongiform agents. Alternatively, a medium which is supplemented with human serum may be used, preferably serum of the recipient and/or the donor of the hematopoietic stem cell progenitors.

The medium used preferably was supplemented with EGF (epidermal growth factor), FGF-2 (fibroblast growth factor) or alternatively FGF-1/1µg/ml heparin, transferrin, selenite, progesterone, putrescine and insulin. Additionally, the medium may contain cytokines.

Human cells and/or tissues of the nervous system or neural stem cell progenitors are provided which are available by the method according to the present invention. It is provided an in vitro cell and/or tissue culture available by the method according to the present invention.

Furthermore, an in vitro cell and/or tissue culture is provided comprising human cells and/or tissues of the nervous system or neural stem cell progenitors generated from human hematopoietic stem cell progenitors. Preferably, the in vitro cell and/or tissue culture comprises human cells and/or tissues of the nervous system or neural stem cell progenitors and a medium containing substances selected from the group: EGF (epidermal growth factor), FGF-2 (fibroblast growth factor) or FGF-1/1µg/ml heparin, transferrin, selenite, progesterone, putrescine and insulin.

It is provided a composition of human cells comprising human hematopoietic stem cell progenitors, which are able to differentiate into cells and/or tissues of the nervous system or neural stem cell progenitors.

The ability to generate non-hematopoietic cells from hematopoietic cells provides new medical applications. Until now for the provision of autologous non-hematopoietic cells for therapy it was necessary to identify cells of the tissue concerned, to isolate and eventually to proliferate or propagate these cells. The present invention now provides a possibility to isolate cells for example from peripheral blood of the person to be treated in order to generate the desired cell type. This method for isolation now is routinously applied in clinics and removes cells from the body which can be regenerated in sufficient numbers in a relatively short time due to the high regenerative potential of the blood system. In

comparison the capacity of self-renewal for regeneration of for example the nervous system or the heart is very limited or does not even exist. For the provision of cells for therapy until now it was necessary to use allogenous cell donors. In the case of neural cells the sources have been fetusses received after abortion. Another possibility was a xenologous transplantation by using cells of porcine origin. In other cases tumor cells which had been irradiated prior transplantation have been used in therapy. All these methods have the disadvantage that the sources of the transplanted cells is a different organism and the risk of rejecting the transplant is very high. This effect can be reduced by the application of immuno-supressive agents which also increase the risk of infection.

The advantage of the inventive method is that the hematopoietic stem cell progenitors can be readily obtained and used for the generation of cells of non-hematopoietic organs and/or tissues for the use in xenogenic, allogenic or autologous transplantation. The present invention also provides compositions containing hematopoietic stem cell progenitors that can be used to generate cells of the nervous system or neural stem cell progenitors. Thus the invention provides a new medical use of hematopoietic stem cell progenitors for the preparation of compositions for augmenting, treating, or altering a patient's neural tissue and/or organs of the nervous system.

The present invention also provides a medicament containing human hematopoietic stem cell progenitors, preferably from peripheral blood. Furthermore, a medicament is provided containing human cells and/or tissues of the nervous system or neural stem cell progenitors generated from human hematopoietic stem cell progenitors. In the case the human cells and/or tissues of the nervous system are generated from a mixed cell population this population originally does not contain cells of the nervous system or neural stem cells or neural stem cell progenitors.

Additionally the present invention provides the use of human hematopoietic stem cell progenitors for the manufacture of a medicament for preventive and/or therapeutic treatment of injuries of the central nervous system, peripheral nervous

system or for preventive and/or therapeutic treatment of neurodegenerative diseases. Neurodegenerative diseases include Alzheimer's Disease, Multiple Sclerosis (MS), Huntington's Disease, Amyotrophic Lateral Sclerosis, and Parkinson's Disease. Damages or injuries of the nervous system include acute brain injury (e.g. stroke, head injury, cerebral palsy) and a large number of CNS dysfunctions (e.g. depression, epilepsy, and schizophrenia). Preferably, for the method of manufacture the human hematopoietic stem cell progenitors are differentiated into cells and/or tissues of the nervous system or neural stem cell progenitors.

The method for the manufacture of said medicament may comprise one ore more of the preferred features of the above described method for generating human cells and/or tissues of the nervous system or neural stem cell progenitors from human hematopoietic stem cell progenitors.

The present invention also provides the use of human cells and/or tissues of the nervous system or neural stem cell progenitors for the manufacture of a medicament for preventive and/or therapeutic treatment of neurodegenerative diseases, wherein the cells and/or tissues of the nervous system or neural stem cell progenitors are generated from human hematopoietic stem cell progenitors. The method for the manufacture of said medicament may comprise one ore more of the preferred features of the above described method for generating human cells and/or tissues of the nervous system or neural stem cell progenitors from human hematopoietic stem cell progenitors.

The method for generating human cells and/or tissues of the nervous system or neural stem cell progenitors from human hematopoietic stem cell progenitors comprises placing the hematopoietic stem cell progenitors in an environment, such as a patient's spinal cord or brain, that induces the hematopoietic stem cell progenitors to differentiate into cells of the nervous system, to produce cells of the nervous system or to differentiate into cells, that correct the neurotransmitter release. The method can be used to treat a patient by restoration of function in neurodegenerative diseases as well as nervous system damages and injuries by

administering cells that can be autologous, allogenic or xenogenic to the patient and/or genetically modified.

More detailed description of the invention

Methods are provided for the generation of human cells and/or tissues of the nervous system or neural stem cell progenitors from hematopoietic stem cell progenitors. The hematopoietic stem cell progenitors may be obtained from various sources including adult bone marrow, cytokine mobilised peripheral blood cells and umbilical cord blood. The immediate progeny of the hematopoietic stem cells is believed to be "progenitor" cells, which are capable of giving rise to various cell types within one or more lineages. The stem or progenitor cells are identified or selected through the use of reagents that specifically bind to the CD133 antigen, e.g. CD133 monoclonal antibody. The high tissue specificity of CD133 expression is particularly advantageous during enrichment for highly purified progenitor cell populations. The CD133 positive cell population is highly enriched for cells that are active in assays measuring progenitor cell activity, particularly in the CFU-GM activity. The CD133 positive cell population is preferably also CD34 positive and/or CD117 positive.

By use of a monoclonal antibody (US patent No. 5,843,633) binding to the CD133 antigen which is expressed on a subset of a hematopoietic progenitor cells derived from human bone marrow, umbilical cord blood and adult peripheral blood these cells can be identified, isolated and enriched. The subset of progenitor cells recognised by CD133 are $CD34^{bright}$, and contain substantially all of the CFU-GM activity present in the $CD34^{+}$ subset. For purposes of transplantation, cells active in CFU-GM are of particular interest because they provide for production of neutrophils. CD133 antibody provides a means for the positive immunoselection of hematopoietic progenitor cell populations using flow cytometry. Cells which are selected for the expression of CD133 antigen may be further purified and/or enriched by selection of other hematopoietic stem cell and progenitor cell markers.

In a preferred embodiment of the present invention the enrichment of the hematopoietic stem cell progenitors is performed by contacting the mixed cell population with a reagent that binds to one of the following antigens: CD34, CD117, CD133, and selecting for cells that have bound for the reagent to produce a population enriched for hematopoietic stem cell progenitors.

Human stem cells have been reported to have the phenotype $CD34^{bright}$; $HLA\text{-}DR^{+}$; $CD38^{dim/negative}$; $CD117(c\text{-}kit)^{dim}$; $CDw90(Thy\text{-}1)^{+}$; and to lack expression of a variety of lineage specific markers, including CD3, CD4, CD7, CD8, CD14 and CD15. A negative designation indicates that the level of staining is at or below the brightness of an isotype matched negative control. A dim designation indicates that the level of staining may be near the level of a negative stain but may also be brighter than an isotype matched control.

Procedures for separation may include magnetic separation, using antibody-coated magnetic beads, affinity chromatography and "panning" with antibody attached to a solid matrix, e.g. plate, or other convenient techniques. Techniques providing accurate separation include fluorescence activated cell sorters, which can have varying degrees of sophistication, such as multiple color channels, low angle and obtuse light scattering detecting channels, impedance channels, etc. Dead cells may be eliminated by selection with dyes associated with dead cells (propidium iodide, LDS). Red blood cells may be removed by elutriation, hemolysis, Ficoll-Paque gradients, etc. Any technique may be employed which is not unduly detrimental to the viability of the selected cells.

Conveniently, the antibodies are conjugated with labels to allow for ease of separation of the particular cell type, e.g. magnetic beads; biotin, which binds with high affinity to avidin or streptavidin; fluorochromes, which can be used with a fluorescence activated cell sorter; haptens; and the like. Multi-color analysis may be employed with the FACS or in a combination of immunomagnetic separation and flow cytometry. Multi-color analysis is of interest for the separation for cells based on multiple surface antigens, e.g. $CD133^{+}$, $CD117^{+}$, $CD34^{+}$. Fluorochromes

which find use in a multi-color analysis include phycobiliproteins, e.g. phycoerythrin and allophycocyanins; fluorescein and Texas red.

The selecting antibody/antibodies may directly or indirectly be conjugated to a magnetic reagent, such as a superparamagnetic microparticle. Direct conjugation to a magnetic particle is achieved by use of various chemical linking groups, as known in the art. Antibody can be coupled to the microparticles through side chain amino or sulfhydryl groups and heterofunctional cross-linking reagents. A large number of heterofunctional compounds are available for linking to entities.

Alternatively, CD133 antibody is indirectly coupled to the magnetic particles. The antibody is directly conjugated to a hapten, and hapten-specific, second stage antibodies are conjugated to the particles. Suitable haptens include digoxin, digoxigenin, FITC (fluoresceinisothiocyanate), dinitrophenyl, nitrophenyl, avidin, biotin, etc. Methods for conjugation of the hapten to a protein, i.e. are known in the art, and kits for such conjugations are commercially available.

The antibody is added to a mixed population of human cells comprising hematopoietic stem cell progenitors. The amount of CD133 antibody to bind a particular cell subset is empirically determined by performing a test separation and analysis. The cells and CD133 antibody are incubated for a period of time sufficient for complexes to form, usually at least about five minutes, more usually at least about ten minutes and usually not more than one hour, more usually not more than about 30 minutes.

The cells may additionally be incubated with antibodies or binding molecules specific for cell surface markers known to be present or absent on hematopoietic stem cell progenitors. For example, CDw90, CD117 and HLA-DR are useful in the positive selection of stem cells. Various markers known to be absent on stem cells, such as CD3, CD4, CD8, CD14, CD15, etc. may be used for negative selection.

The labeled cells are separated in accordance with a specific antibody preparation. Fluorochrome labeled antibodies are useful for FACS separation, magnetic particles for immunomagnetic selection, particularly high gradient magnetic selection (HGMS), etc. Exemplary magnetic separation devices are described in WO/90/07380, PCT/US96/00953 and EP 438,520, herein incorporated by reference.

The purified cell population may be collected in any appropriate medium. Various media are commercially available and may be used, including Dulbecco's Modified Eagle Medium (DMEM), Hank's Basic Salt Solution (HBSS), Dulbecco's phosphate buffered saline (DPBS), RPMI, Iscove's modified Dulbecco's medium (IMDM), phosphate buffered saline (PBS) with 5 mM EDTA, etc., frequently supplemented with bovine serum albumin (BSA), human serum albumin (HSA), etc.

Compositions which are highly enriched for human hematopoietic stem cell progenitors are achieved in this manner. After the enrichment the desired cells will be at least about 5 % of the cells present having the hematopoietic stem cell progenitors phenotype, preferably at least about 10 % of the cells present, more preferred about 60 % and most prefered about 90 % or more of the cell composition. Preferably the cell population contains at least 90 % of cells expressing the cell surface marker antigen CD45.

Specific populations of interest include $CD133^+$ cells, which are characterised as $CD34^{bright}$ and $HLA\text{-}DR^+$. The cell population used may contain at least 10 % of cells expressing the cell surface marker antigen CD133. This population may be further selected for those cells that are $CDw90^+$, CD117 and/or $CD38^{dim}$. Functionally these cells are highly enriched for CFU-GM activity. According to this invention cells expressing one or more of the cell surface antigens CD133, CD117, CD34 will be used. Each desired hematopoietic stem cell progenitors express one or more of the cell surface marker antigens selected from the group CD45, CD34, CD133 and CD117. Another population of interest is $CD133^-$ and $CD34^+$, which is enriched for BFU-e activity.

For maintaining the hemotopoietic stem cell progenitor characteristics the desired cells which have been isolated, may be propagated by growing in conditioned medium from stromal cells, coculturing with such stromal cells, or in medium, comprising maintenance factors supporting the proliferation of such progenitor cells.

For maintainance the nature of hemotopoietic stem cell progenitors according to the invention CD133 positive cells are cultivated at a temperature of 37 °C under 5 % CO_2-atmosphere for several days, weeks or months, preferably 2 – 15 weeks, most preferably for about 10 weeks. For the cultivation of the isolated cells media on the basis of basal media formulations like DMEM or RPMI formulations have been used which are supplemented with specific proteins or protein fragments. As specific proteins, cytokines and mixtures of cytokines, respectively, have been used. These cytokines may also be replaced by supernatants of stromal cell cultures. Preferably, cytokines such as EGF (epidermal growth factor) and FGF-2 (fibroblast growth factor) or alternatively FGF-1/1µg/ml heparin are used.

Preferably IL-6 (interleukin 6) and Flt (fetal liver tyrosinkinase)–3 – ligand was added to the medium RPMI-1640 (Gibco-BRL) each in a concentration of 50 to 200 ng/ml, preferably about 100 ng/ml. The cultivation was repeated under the same conditions with the difference that 1 % (v/v) of autologous plasma was added. After seven days adherent cells could be detected. After 12 days of cultivation the amount of adherent cells was determined to be >12 % under the described conditions. The proliferation of the cells occurred with a proliferation rate of about 6 – 7 times per 3 weeks indicating an average generation time of 3 days.

For the induction of the differentiation of the hematopoietic stem cell progenitors to human cells and/or tissues of the nervous system or to neural stem cell progenitors a medium is added to the cell population containing one or more predetermined growth factors effective for inducing differentiation into cells and/or tissues of the nervous system or into neural stem cell progenitors.

For inducing the isolated cells to differentiate they are cultivated under standard conditions (37 °C, 5 % CO_2) for several days, weeks or months, preferably 2 – 6 week, most preferably for about 30 days. For the cultivation media on the basis of well-known DMEM- (Dulbecco's Modified Eagle's Medium) or RPMI-formulations are added. These media are supplemented with specific proteins or protein fragments. As specific proteins cytokines or mixtures of different cytokines may be been used. These cytokines may also be replaced by supernatants of stromal cell cultures. Preferably, as cytokines EGF (epidermal growth factor) and FGF-2 (fibroblast growth factor) or alternaltively FGF-1 / 1µg/ml heparin are used.

A typical media formulation for inducing and maintaining differentiation of the cells contains 1 – 50 ng/ml EGF (epidermal growth factor), 1 – 50 ng/ml FGF-2 (fibroblast growth factor) 1 – 10 µg/ml insulin, 10 – 200 µg/ml human transferrin, 1 – 10 ng/ml progesterone, 1 – 20 µg/ml putrescine, and/or 1 – 10 ng/ml selenite. The cytokines (EGF and FGF-2 or FGF-1 / 1µg/ml heparin) may be replaced by supernatants of T-cell or B-cell cultures or cultures of stromal cells.

Preferably, DMEM medium (Dulbecco's Modified Eagle's Medium) supplemented with EGF (epidermal growth factor), FGF-2 (fibroblast growth factor) each with a concentration of 20 ng/ml, and B27 of GIBCO BRL in a ratio of 1 : 100 have been used for cultivation of the cells under conditions of differentiation.

Alternatively, said medium was supplemented with a mixture of EGF (epidermal growth factor), FGF-2 (fibroblast growth factor) each with a concentration of 20 ng/ml, human transferrin (concentration: 100 µg/ml), selenite (5.2 ng/ml), progesterone (6.3 ng/ml), putrescine (16.11 µg/ml) and insulin (5 µg/ml).

During this culture the cells differentiated into cells of the nervous system as showed by antibody staining of specific antigens of the nervous system like GFAP (glial fibrillary acidic protein) as a marker for astrocytes or β-tubulin III and neurofilament 70 as markers for neurons. After 30 days of cultivation under conditions of differentiation up to 90 % or more of viable cells show the characteristics of one of the cells of the nervous system. These differentiated cells

are identified to be one of the following cells: neurons, glial cells which are either astrocytes, oligodendrocytes or Schwann cells. At a certain time point after induction of differentiation the cells are $CD45^-$, indicating that the differentiation from $CD45^+$ hematopoietic stem cells was completed.

The hematopoietic stem cell progenitors or the non-hematopoietic cells derived from them are useful in a variety of uses. These cells can be used to reconstitute a host whose cells have been lost trough disease or injury. Genetic diseases associated with cells may be treated by genetic modification of autologous or allogeneic stem cells to correct a genetic defect or treat to protect against diseases. Alternatively, normal allogeneic progenitor cells may be transplanted. CNS disorders encompass numerous afflictions such as neurodegenerative diseases (e.g. Alzheimer's and Parkinson's), acute brain injury (e.g. stroke, head injury, cerebral palsy) and a large number of CNS dysfunctions (e.g. depression, epilepsy, and schizophrenia). In recent years neurodegenartive diseases have become an important concern due to the expanding elderly population which is at greatest risk for these disorders. Theses diseases, which include Alzheimer's Disease, Multiple Sclerosis (MS), Huntington's Disease, Amyotrophic Lateral Sclerosis, and Parkinson's Disease, have been linked to the degeneration of neural cells in particular locations of the CNS, leading to the inability of these cells or the brain region to carry out their intended function.

The human hematopoietic stem cell progenitors or the cells which are generated from them may be used for reconstitution of organ function in a recipient preferably the reconstitution of the nervous system. Therefore the invention also provides a medicament containing hematopoietic stem cell progenitors from peripheral blood. In a further embodiment the medicament is containing non-hematopoietic stem cells and tissues respectively generated from a cell population comprising human hematopoietic stem cell progenitors, wherein the non-hematopoietic cells comprise cells selected from the group: neurons, glial cells, Schwann cells, astrocytes, oligodendrocytes.

In a further embodiment the hematopoietic stem cell progenitors from peripheral blood are used for the manufacture of a medicament for preventive and/or therapeutic treatment of injuries of the central nervous system, peripheral nervous system or for preventive and/or therapeutic treatment of neurodegenerative diseases. Furthermore non-hematopoietic cells which are generated from a cell population comprising human hematopoietic stem cell progenitors are used for the manufacture of a medicament for preventive and/or therapeutic treatment of injuries of the nervous system or for preventive and/or therapeutic treatment of neurodegenerative diseases. In a particularly preferred embodiment the non-hematopoietic cells comprise cells selected from the group: neurons, glial cells, schwan cells, astrocytes, oligodendrocytes.

The present invention also provides a method for preventive and/or therapeutic treatment of injuries of the central nervous system or peripheral nervous system or for preventive and/or therapeutic treatment of neurodegenerative diseases, wherein human cells and/or tissues of the nervous system or neural stem cell progenitors are produced from hematopoietic stem cell progenitors comprising the following steps:

a) obtaining a mixed population of human cells comprising hematopoietic stem cell progenitors from peripheral blood and/or bone marrow and/or cord blood;

b) enrichment of the hematopoietic stem cell progenitors by selecting by those cells that are CD34+ and/or CD117+ and/or CD133+,
and additionally to the enrichment step or instead of the enrichment step: depletion of those cells that are in a more differentiated state than the hematopoietic stem cell progenitors;

c) induction of the differentiation of hematopoietic stem cell progenitors to non-hematopoietic cells by placing the cell population comprising enriched hematopoietic stem cell progenitors in an appropriate cell inducing environment,

wherein the cells are introduced into the recipient before or after the induction of the differentiation.

The method may comprise one ore more of the preferred features of the above described method for generating human cells and/or tissues of the nervous system or neural stem cell progenitors from human hematopoietic stem cell progenitors.

Description of the figures

Fig. 1: shows CD45-FITC labelled cells plotted versus granularity ("Side Scatter") before separation step

Fig. 2: shows Propidium Iodide stained cells versus AC133-PE labelling before separation step

Fig. 3: shows CD34-APC staining of CD45+ Cells before separation step

Fig. 4: shows CD45 labelled cells versus granularity of CD34+ Cells before separation step

Fig. 5: shows light scatter characteristics of CD34+ cells before separation step

Fig. 6: shows AC133/1-PE labelled cells versus CD34-APC before separation step

Fig. 7: shows CD45-FITC labelled cells plotted versus granularity ("Side Scatter") after separation step

Fig. 8: shows Propidium Iodide stained cells versus AC133-PE labelling after separation step

Fig. 9: shows CD34-APC staining of CD45+ Cells after separation step

Fig. 10: shows CD45 labelled cells versus granularity of CD34+ Cells after separation step

Fig. 11: shows light scatter characteristics of CD34+ cells after separation step

The figures are described in more detail in Example 2. The term AC133 used in the figures is a former name of the CD133 antigen. AC133 has therefore the same meaning as CD133.

The following examples are offered by way of illustration and not by way of limitation.

Examples

Example 1: Isolation of hematopoietic stem cell progenitors

CD133 positive cells have been isolated by magnetic labelling of the target cells and subsequent separation by using an automated cell separation process (MACS technology). The amount of white blood cells has been determined to be $4x10^9$. The product of the leukapheresis has been adjusted to a volume of 600 ml with PBS/EDTA buffer supplemented with 0,5 % human serum albumin. The cell suspension was subjected to a centrifugation step for 15 min at room temperature at 200 x g. The supernatant was discarded and the sample was adjusted to a final volume of 60 ml. 5 ml FcR blocking reagent has been added afterwards. The suspension was added with 7,8 ml CD133 MicroBeads, a reagent labelling CD133 positive cells. The sample was incubated by using an orbital shaker (at 25 RPM for 30 minutes at room temperature). The cell suspension was adjusted to volume of 600 ml with PBS/EDTA buffer supplemented with 0,5 % human serum albumin. Then the cell suspension was subjected to a centrifugation step for 15 minutes at room temperature at 200 x g. The supernatant was discarded, the pellet was resuspended and the volume was subsequently adjusted to 100 ml with CliniMACS® PBS/ETA buffer (Miltenyi Biotec) supplemented with 0,5 % human serum albumin. CD133 positive cells have been isolated from all the other cells by use of a cell separator (CliniMACS®, Miltenyi Biotec). Subsequently the cells have been assayed for the expression of CD45, CD133 and CD34 antigens.

Example 2: Analysis of the cells

For the analysis of the isolated CD133 positive cells the amount of target cells is calculated by determining a percentage of the leukapheresis product compared to the amount of total cells. The analysis of CD133 positive (=AC133+) cells is based on labelling the cells with fluorochrome conjugated monoclonal antibodies and

subsequently determination by flow cytometry. For the analysis samples of 0.5 ml are taken from the leukapheresis product prior and after the separation.

Analysis of CD133 positive cells before separation

Figures 1 to 6 show the enumeration of CD133+ cells in apheresis sample before preparation using AC133/1-PE / CD34-APC and CD45-FITC labelling (PE = phycoerythrin; APC = allophycoerythrin; FITC = fluoresceinisothiocyanate). Precise details of gating strategy are described in the text above. Analysis of CD133 positive progenitor cells was performed by fluorochrome conjugated monoclonal antibody labeling of the target cells followed by a determination using a flow cytometer. Analysis of all samples were performed according to the ISHAGE guidelines for CD34 positive cell determination by flow cytometry. The ISHAGE guidelines are basing on a gating strategy to select the population of interest and simultaneously minimize interference from debris and mature cells to which antibodies can bind non-specifically.

Analysis of CD133 positive cells after separation

Cells stained with CD34-APC and CD45-FITC were analyzed using identical gates defined by R1-R5 for the first three "Before preparation" samples. To calculate the purity and absolute number of viable CD133+ cells in the positive fraction the corrected number of CD34+ cells gated in region G5 is divided by the number of viable white blood cells (WBCs) (gated in gating region 2 = R1*R2). This value is multiplied by 100. To calculate the total number of viable CD133 stem/progenitor cells in the samples the purity is multiplied by the total number of viable WBCs.

Figures 7 to 11 show the enumeration of CD34+ cells in apheresis sample after CliniMACS® separation (Positive Fraction) using CD45-FITC / CD34-APC. Precise details of gating strategy are described in the text above.

Fig. 1 and Fig. 7 show CD45-FITC labelled cells plotted versus granularity ("Side Scatter") before (Fig. 1) and after (Fig. 7) separation step. The purpose of these

plots is to exclude CD45-negative cells, because the target cell population is CD45-antigen positive. Therefore CD45 (log FL1) versus side scatter (lin SSC) of all events were displayed. Gating region R1 included all events except those that are CD45 negative.

Fig. 2 and Fig. 8 show Propidium Iodide stained cells versus AC133-PE labelling before (Fig. 2) and after (Fig. 8) separation step. The purpose of these plots is to exclude dead cells, because the target cell population is a living cell population. PI (propidium iodide) stains only dead cells by intercalation into nucleic acids. Therefore Propidium Iodide (log FL3) versus AC133 (log FL2) of all CD45+ events derived from primary gating region 1 (R1) was displayed. The region R2 included all viable WBCs (white blood cells).

Fig. 3 and Fig. 9 show shows CD34-APC staining of CD45+ cells before (Fig. 3) and after (Fig. 9) separation step. The purpose of these plots is to exclude CD34-negative cells, because the target cell population is nearly CD34-Antigen positive. CD34-APC (log FL4) versus side scatter (lin SSC) is displayed to gate potential CD34+ events from gating region 2 (R1*R2).

Fig. 4 and Fig. 10 show CD45 labelled cells versus granularity of CD34+ cells before (Fig. 4) and after (Fig. 10) separation step. In Fig. 4 CD34+ cells from additive regions R1 (Fig. 1), R2 (Fig. 2) and R3 (Fig. 3) forming a cluster with characteristic low SSC and dim CD45 fluorescence are gated by region R4. Non-specifically stained events were excluded from this region.

Fig. 5 and 11 show light scatter characteristics of CD34+ cells before (Fig. 5) and after (Fig. 11) separation step. Forward scatter versus side scatter of cells from additive regions R1 (Fig. 1), R2 (Fig. 2), R3 (Fig. 3) and R4 (Fig. 4) were displayed. The lymph/blast region R5 identifies a cluster of events meeting all the fluorescence and light scatter criteria of CD34+ stem/progenitor cells. Cells clustered in region R5 exhibit slightly higher forward scatter than that of small lymphocytes and uniformly low side scatter. Any events falling outside of region R5 are not included in the percentage of viable CD34+ cells calculation.

Fig. 6 shows AC133/1-PE labelled cells versus CD34-APC before separation step. AC133-PE (log FL2) versus CD34-APC (log FL4) on all viable CD34+ cells derived from primary gating region 5 (R1*R2*R3*R4*R5) were displayed. The CD133+ population is shown to constitute a specific subpopulation of CD34+ cells gated in region R6.

The gate statistic of these dot plots is displayed in tables 1 and 2.

Table 1: enumeration of the cells before separation

	% gated	% total
white blood cells (G1=R1)	100,00	99.05
viable WBCs (G2=R1*R2)	94.59	93.70
G3=R1*R2*R3	0.27	0.27
G4=R1*R2*R3*R4	0.21	0.21
viable CD34+ cells (G5=R1*R2*R3*R4*R5)	0.21	0.21
AC133+/CD34+ cells (G5=R1*R2*R3*R4*R5*R6)	0.12	0.12

Table 2: enumeration of the cells after separation

	% gated	% total
white blood cells (G1=R1)	100,00	88.54
viable WBCs (G2=R1*R2)	98.67	87.36
G3=R1*R2*R3	90.58	80.20
G4=R1*R2*R3*R4	90.42	80.06
viable CD34+ cells (G5=R1*R2*R3*R4*R5)	90.42	80.06

Example 3: Proliferation of the cells

CD133 positive cells are cultivated at a temperature of 37 °C under 5 % CO_2-atmosphere for 10 weeks. For the cultivation of the isolated cells media on the basis of basal media formulations like DMEM or RPMI (Roswell Park Memorial Institute) formulations have been used which are supplemented with specific proteins or protein fragments. As specific proteins, cytokines and mixtures of cytokines, respectively, have been used. The cytokines may also be replaced by supernatants of T-cell or B-cell cultures or cultures of stromal cells.

IL-6 (interleukin 6) and Flt (fetal liver tyrosinkinase)–3 – ligand was added to the medium RPMI-1640 (Gibco-BRL) in a concentration of each 100 ng/ml. The cultivation was repeated under the same conditions with the difference that 1 % (v/v) of autologous plasma was added. After seven day adherent cells could be detected. After 12 days of cultivation the amount of adherent cells was determined to be >12 % under the described conditions. The proliferation of the cells occurred with a proliferation rate of about 6 – 7 times per 3 weeks indicating an average generation time of 3 days. The cells were tested for the expression of CD133 and CD134 antigens.

Example 4: Differentiation to neural cells

The mixed cell population containing 64.9 % CD133 positive cells was isolated from peripheral blood by MACS-technology. In Table 3 the analysis of the cell surface markers is summarised.

Table 3: percentage of the cells expressing different cell surface antigens.

cell surface antigen	percentage of positive cells prior separation	percentage of positive cells after separation
CD34	0.8 %	64.9 %
CD45	99.0 %	99.9 %
CD133	0.04 %	64.9 %

For differentiation the isolated cells are cultivated under standard conditions (37 °C, 5 % CO_2) for 30 days. As a medium for the cultivation of the isolated cells media on the basis of well-known DMEM- or RPMI-formulations have been used supplemented with specific proteins or protein fragments. As specific proteins cytokines or mixtures of different cytokines are used. These cytokines may also be replaced by supernatants of stromal cell cultures. Cytokines such as EGF (epidermal growth factor) and FGF-2 (fibroblast growth factor) or alternatively FGF-1 / 1µg/ml heparin are used.

DMEM medium (Dulbecco's Modified Eagle's Medium) was supplemented with EGF (epidermal growth factor), FGF-2 (fibroblast growth factor) each with a concentration of 20 ng/ml, and B27 of GIBCO BRL in a ratio of 1 – 100. Alternatively, DMEM medium was supplemented with a mixture of EGF (epidermal growth factor), FGF-2 (fibroblast growth factor) human transferrin (concentration: 100 µg/ml), selenite (5.2 ng/ml), progesterone (6.3 ng/ml), putrescine (16.11 µg/ml) and insulin (5 µg/ml).

The cells differentiated into cells of the nervous system. The development of cell differentiation was verified by use of fluorescence labeled antibodies directed against proteins specific for cells of the nervous tissue: antibodies directed against GFAP (glial fibrillary acidic protein) as a marker for astrocytes and beta-tubulin III; antibodies directed against neurofilament 70 as a marker for neurons.

As a result after 30 days about 90 % of all viable cells present in the in vitro culture showed the characteristics of cells of the nervous system. The cells are identified as neurons, glial cells, Schwann cells, astrocytes, oligodendrocytes. None of the cells showed any characteristics of blood cells anymore, as shown by the non-expression of CD45-antigen.

Claims

1. A method for the generation of human cells and/or tissues of the nervous system or neural stem cell progenitors from human hematopoietic stem cell progenitors.

2. The method according to claim 1, wherein the generated human cells and/or tissues comprise cells selected from the group neurons, glial cells, Schwann cells, astrocytes, oligodendrocytes.

3. The method according to claim 1 or 2, wherein the human hematopoietic stem cell progenitors are isolated from peripheral blood and/or bone marrow and/or umbilical cord blood.

4. The method according to any of claims 1 to 3, wherein the human hematopoietic stem cell progenitors are cultivated and/or propagated in vitro after isolation.

5. The method according to any of claims 1 to 4, wherein for the generation of human cells and/or tissues of the nervous system or neural stem cell progenitors a population of cells is used wherein at least about 5 % of the cells present having the hematopoietic stem cell progenitors phenotype, preferably at least about 10 % of the cells present.

6. The method according to any of claims 1 to 5, wherein for the generation of human cells and/or tissues of the nervous system or neural stem cell progenitors a cell population is used containing cells expressing the cell surface marker antigen CD45.

7. The method according to any of claims 1 to 6, wherein for the generation of human cells and/or tissues of the nervous system or neural stem cell progenitors a cell population is used containing at least 10 % of cells expressing the cell surface marker antigen CD133.

8. The method according to any of claims 1 to 7, comprising the steps of:

a) obtaining a mixed population of human cells comprising hematopoietic stem cell progenitors from peripheral blood and/or bone marrow and/or umbilical cord blood,

b) enrichment of the hematopoietic stem cell progenitors by selecting for those cells that are CD34 positive and/or CD117 positive and/or CD 133 positive;

and additionally to the enrichment step or instead of the enrichment step: depletion of those cells that are in a more differentiated state than the hematopoietic stem cell progenitors;

c) induction of the differentiation of the hematopoietic stem cell progenitors to cells and/or tissues of the nervous system or neural stem cell progenitors by placing the cell population comprising enriched hematopoietic stem cell progenitors in an appropriate cell-inducing environment.

9. The method according to claim 8, wherein the enrichment in step b) is performed by
contacting the mixed cell population with one or more reagents that bind to antigens selected from the group: CD34, CD117, CD133; and
selecting for cells that have bound the reagent, to produce a population enriched for hematopoietic stem cell progenitors.

10. The method according to claim 8 or 9, wherein in the depletion step cells are removed having cells surface antigens selected from the group: CD2, CD3, CD14, CD16, CD19, CD24, CD56, CD66b, Glycophorin A.

11. The method according to any of claims 8 to 10, wherein after the enrichment the cell populations are at least about 5 % of the cells present having the hematopoietic stem cell progenitors phenotype, preferably at least about 10 % of the cells present.

12. The method according to any of claims 8 to 11, wherein for the induction of the differentiation the cells are cultivated in a medium supplemented with growth factors which induce the differentiation of human hematopoietic stem cell progenitors into cells of the nervous system or neural stem cell progenitors.

13. The method according to any of claims 8 to 12, wherein for the induction of the differentiation the cells are cultivated in a medium supplemented with compounds selected from the group: EGF (epidermal growth factor), FGF-2 (fibroblast growth factor), FGF-1 with heparin, transferrin, selenite, progesterone, putrescine, insulin.

14. The method according to any of claims 8 to 13, wherein for the induction of the differentiation the cells are cultivated in a medium which is serum free.

15. The method according to any of claims 8 to 13, wherein for the induction of the differentiation the cells are cultivated in a medium which is supplemented with human serum.

16. The method according to any of claims 8 to 11, wherein the induction of the differentiation occurs in vivo.

17. Human cells and/or tissues of the nervous system or neural stem cell progenitors available by the method according to any of claims 1 to 16.

18. An in vitro cell and/or tissue culture available by the method according to any of claims 1 to 16.

19. An in vitro cell and/or tissue culture comprising human cells and/or tissues of the nervous system or neural stem cell progenitors generated from human hematopoietic stem cell progenitors.

20. The in vitro cell and/or tissue culture according to claim 19, comprising human cells and/or tissues of the nervous system or neural stem cell progenitors and a medium containing substances selected from the group: EGF (epidermal growth factor), FGF-1 with heparin, FGF-2 (fibroblast growth factor), transferrin, selenite, progesterone, putrescine and insulin.

21. A composition of human cells comprising human hematopoietic stem cell progenitors, which are able to differentiate into cells and/or tissues of the nervous system or neural stem cell progenitors.

22. Medicament containing human hematopoietic stem cell progenitors.

23. Medicament containing human cells and/or tissues of the nervous system or neural stem cell progenitors generated from human hematopoietic stem cell progenitors.

24. Use of human hematopoietic stem cell progenitors for the manufacture of a medicament for preventive and/or therapeutic treatment of injuries of the central nervous system, peripheral nervous system or for preventive and/or therapeutic treatment of neurodegenerative diseases.

25. The use of claim 24, wherein the human hematopoietic stem cell progenitors are differentiated into cells and/or tissues of the nervous system or into neural stem cell progenitors according to any method of claims 1 to 16.

26. Use of human cells and/or tissues of the nervous system or neural stem cell progenitors for the manufacture of a medicament for preventive and/or therapeutic treatment of injuries of the central nervous system, peripheral

nervous system or for preventive and/or therapeutic treatment of neurodegenerative diseases, wherein the human cells and/or tissues of the nervous system or neural stem cell progenitors are generated from human hematopoietic stem cell progenitors according to any method of claims 1 to 16.

27. A method for the the propagation and maintainance of human hematopoietic stem cell progenitors, wherein said cells are propagated under conditions selected from the group:
    a) growing said cells in conditioned medium from stromal cells,
    b) coculturing said cells with stromal cells,
    c) growing said cells in medium comprising maintenance factors supporting the proliferation of human hematopoietic stem cell progenitors.

28. The method of claim 27, wherein the hemotopoietic stem cell progenitors are cultivated on the basis of basal media formulations which are supplemented with substances selected from the group cytokines, mixtures of cytokines, supernatants of stromal cell cultures, EGF (epidermal growth factor), FGF-2 (fibroblast growth factor), FGF-1 with heparin.

29. The method of claim 27 or 28, wherein as maintenance factors IL-6 (interleukin 6) and Flt (fetal liver tyrosinkinase)–3 – ligand is added to the media each in a concentration of 50 to 200 ng/ml, preferably of about 100 ng/ml.

30. The method of any one of claims 27 to 29, wherein the cultivation is repeated under the same conditions and in presence of about 1 % (v/v) of autologous plasma.

Claims for US

1. A method for preventive and/or therapeutic treatment of injuries of the central nervous system, peripheral nervous system or for preventive and/or therapeutic treatment of neurodegenerative diseases, wherein human cells of the nervous system or neural stem cell progenitors are produced from hematopoietic stem cell progenitors, and the cells are introduced into the recipient before or after the induction of the differentiation.

2. The method according to claim 1, wherein the generated human cells and/or tissues comprise cells selected from the group neurons, glial cells, Schwann cells, astrocytes, oligodendrocytes.

3. The method according to claim 1, wherein the human hematopoietic stem cell progenitors are isolated from peripheral blood and/or bone marrow and/or umbilical cord blood.

4. The method according to claim 1, wherein the human hematopoietic stem cell progenitors are cultivated and/or propagated in vitro after isolation.

5. The method according to claim 1, wherein for the generation of human cells and/or tissues of the nervous system or neural stem cell progenitors a population of cells is used wherein at least about 5 % of the cells present having the hematopoietic stem cell progenitors phenotype, preferably at least about 10 % of the cells present.

6. The method according to claim 1, wherein for the generation of human cells and/or tissues of the nervous system or neural stem cell progenitors a cell population is used containing cells expressing the cell surface marker antigen CD45.

7. The method according to claim 1, wherein for the generation of human cells and/or tissues of the nervous system or neural stem cell progenitors a cell

population is used containing at least 10 % of cells expressing the cell surface marker antigen CD133.

8. The method according to claim 1, comprising the steps of:

a) obtaining a mixed population of human cells comprising hematopoietic stem cell progenitors from peripheral blood and/or bone marrow and/or umbilical cord blood,

b) enrichment of the hematopoietic stem cell progenitors by selecting for those cells that are CD34 positive and/or CD117 positive and/or CD 133 positive,

and additionally to the enrichment step or instead of the enrichment step: depletion of those cells that are in a more differentiated state than the hematopoietic stem cell progenitors;

c) induction of the differentiation of the hematopoietic stem cell progenitors to cells and/or tissues of the nervous system or neural stem cell progenitors by placing the cell population comprising enriched hematopoietic stem cell progenitors in an appropriate cell-inducing environment.

9. The method according to claim 8, wherein the enrichment in step b) is performed by
contacting the mixed cell population with one or more reagents that bind to antigens selected from the group: CD34, CD117, CD133; and
selecting for cells that have bound the reagent, to produce a population enriched for hematopoietic stem cell progenitors.

10. The method according to claim 8, wherein in the depletion step cells are removed having cells surface antigens selected from the group: CD2, CD3, CD14, CD16, CD19, CD24, CD56, CD66b, Glycophorin A.

11. The method according to claim 8, wherein after the enrichment the cell populations are at least about 5 % of the cells present having the hematopoietic stem cell progenitors phenotype, preferably at least about 10 % of the cells present.

12. The method according to claim 8, wherein for the induction of the differentiation the cells are cultivated in a medium supplemented with growth factors which induce the differentiation of human hematopoietic stem cell progenitors into cells of the nervous system or neural stem cell progenitors.

13. The method according to claim 8, wherein for the induction of the differentiation the cells are cultivated in a medium supplemented with compounds selected from the group: EGF (epidermal growth factor), FGF-2 (fibroblast growth factor), FGF-1 with heparin, transferrin, selenite, progesterone, putrescine, insulin.

14. The method according to claim 8, wherein for the induction of the differentiation the cells are cultivated in a medium which is serum free.

15. The method according to claim 8, wherein for the induction of the differentiation the cells are cultivated in a medium which is supplemented with human serum.

16. The method according to claim 8, wherein the induction of the differentiation occurs in vivo.

17. A method for preventive and/or therapeutic treatment of injuries of the central nervous system, peripheral nervous system or for preventive and/or therapeutic treatment of neurodegenerative diseases, wherein human cells and/or tissues of the nervous system or neural stem cell progenitors are produced from hematopoietic stem cell progenitors and introduction into the

recipient before or after the induction of the differentiation, comprising the steps of:

a) obtaining a mixed population of human cells comprising hematopoietic stem cell progenitors from peripheral blood and/or bone marrow and/or umbilical cord blood,

b) enrichment of the hematopoietic stem cell progenitors by selecting for those cells that are CD34 positive and/or CD117 positive and/or CD 133 positive,

and additionally to the enrichment step or instead of the enrichment step: depletion of those cells that are in a more differentiated state than the hematopoietic stem cell progenitors;

c) induction of the differentiation of the hematopoietic stem cell progenitors to cells and/or tissues of the nervous system or neural stem cell progenitors by placing the cell population comprising enriched hematopoietic stem cell progenitors in an appropriate cell-inducing environment;

wherein, the hematopoietic stem cell progenitors or the cells and/or tissues of the nervous system or neural stem cell progenitors generated from them are introduced into the recipient before or after the induction of the differentiation.

18. A method for preventive and/or therapeutic treatment of injuries of the central nervous system, peripheral nervous system or for preventive and/or therapeutic treatment of neurodegenerative diseases, wherein human cells and/or tissues of the nervous system or neural stem cell progenitors are produced from hematopoietic stem cell progenitors and introduction into the recipient before or after the induction of the differentiation, comprising the steps of:

a) obtaining a mixed population of human cells comprising hematopoietic stem cell progenitors from peripheral blood and/or bone marrow and/or umbilical cord blood,

b) enrichment of the hematopoietic stem cell progenitors by selecting for those cells that are CD34 positive and/or CD117 positive and/or CD 133 positive by contacting the mixed cell population with one or more reagents that bind to antigens selected from the group: CD34, CD117, CD133, and selecting for cells that have bound the reagent, to produce a population enriched for hematopoietic stem cell progenitors.

and additionally to the enrichment step or instead of the enrichment step: depletion of those cells that are in a more differentiated state than the hematopoietic stem cell progenitors, wherein cells are removed having cells surface antigens selected from the group: CD2, CD3, CD14, CD16, CD19, CD24, CD56, CD66b, Glycophorin A;

c) induction of the differentiation of the hematopoietic stem cell progenitors to cells and/or tissues of the nervous system or neural stem cell progenitors by placing the cell population comprising enriched hematopoietic stem cell progenitors in an appropriate cell-inducing environment;

wherein, the hematopoietic stem cell progenitors or the cells and/or tissues of the nervous system or neural stem cell progenitors generated from them are introduced into the recipient before or after the induction of the differentiation.

19. A method for preventive and/or therapeutic treatment of injuries of the central nervous system, peripheral nervous system or for preventive and/or therapeutic treatment of neurodegenerative diseases, wherein human cells and/or tissues of the nervous system or neural stem cell progenitors are

produced from hematopoietic stem cell progenitors and introduction into the recipient before or after the induction of the differentiation, comprising the steps of:

a) obtaining a mixed population of human cells comprising hematopoietic stem cell progenitors from peripheral blood and/or bone marrow and/or umbilical cord blood,

b) enrichment of the hematopoietic stem cell progenitors by selecting for those cells that are CD34 positive and/or CD117 positive and/or CD 133 positive by contacting the mixed cell population with one or more reagents that bind to antigens selected from the group: CD34, CD117, CD133, and selecting for cells that have bound the reagent, to produce a population enriched for hematopoietic stem cell progenitors.

and additionally to the enrichment step or instead of the enrichment step: depletion of those cells that are in a more differentiated state than the hematopoietic stem cell progenitors, wherein cells are removed having cells surface antigens selected from the group: CD2, CD3, CD14, CD16, CD19, CD24, CD56, CD66b, Glycophorin A;

c) induction of the differentiation of the hematopoietic stem cell progenitors to cells and/or tissues of the nervous system or neural stem cell progenitors by placing the cell population comprising enriched hematopoietic stem cell progenitors in an appropriate cell-inducing environment, wherein for the induction of the differentiation the cells are cultivated in a medium supplemented with growth factors which induce the differentiation of human hematopoietic stem cell progenitors into cells of the nervous system or neural stem cell progenitors;

wherein, the hematopoietic stem cell progenitors or the cells and/or tissues of the nervous system or neural stem cell progenitors generated

from them are introduced into the recipient before or after the induction of the differentiation.

20. A method for preventive and/or therapeutic treatment of injuries of the central nervous system, peripheral nervous system or for preventive and/or therapeutic treatment of neurodegenerative diseases, wherein human cells and/or tissues of the nervous system or neural stem cell progenitors are produced from hematopoietic stem cell progenitors and introduction into the recipient before or after the induction of the differentiation, comprising the steps of:

a) obtaining a mixed population of human cells comprising hematopoietic stem cell progenitors from peripheral blood,

b) enrichment of the hematopoietic stem cell progenitors by selecting for those cells that are CD34 positive and/or CD117 positive and/or CD 133 positive by contacting the mixed cell population with one or more reagents that bind to antigens selected from the group: CD34, CD117, CD133, and selecting for cells that have bound the reagent, to produce a population enriched for hematopoietic stem cell progenitors;

and additionally to the enrichment step depletion of those cells that are in a more differentiated state than the hematopoietic stem cell progenitors, wherein cells are removed having cells surface antigens selected from the group: CD2, CD3, CD14, CD16, CD19, CD24, CD56, CD66b, Glycophorin A;

c) induction of the differentiation of the hematopoietic stem cell progenitors to cells and/or tissues of the nervous system or neural stem cell progenitors by placing the cell population comprising enriched hematopoietic stem cell progenitors in an appropriate cell-inducing environment, wherein for the induction of the differentiation the cells are cultivated in a medium supplemented with compounds

selected from the group: EGF (epidermal growth factor), FGF-2 (fibroblast growth factor), FGF-1 with heparin, transferrin, selenite, progesterone, putrescine, insulin;
wherein, the hematopoietic stem cell progenitors or the cells and/or tissues of the nervous system or neural stem cell progenitors generated from them are introduced into the recipient before or after the induction of the differentiation.

21. A method for the generation of human cells and/or tissues of the nervous system or neural stem cell progenitors from human hematopoietic stem cell progenitors.

22. The method according to claim 21, wherein the generated human cells and/or tissues comprise cells selected from the group neurons, glial cells, Schwann cells, astrocytes, oligodendrocytes.

23. The method according to claim 21, wherein the human hematopoietic stem cell progenitors are isolated from peripheral blood and/or bone marrow and/or umbilical cord blood.

24. The method according to claim 21, wherein the human hematopoietic stem cell progenitors are cultivated and/or propagated in vitro after isolation.

25. The method according to claim 21, wherein for the generation of human cells and/or tissues of the nervous system or neural stem cell progenitors a population of cells is used wherein at least about 5 % of the cells present having the hematopoietic stem cell progenitors phenotype, preferably at least about 10 % of the cells present.

26. The method according to claim 21, wherein for the generation of human cells and/or tissues of the nervous system or neural stem cell progenitors a cell population is used containing cells expressing the cell surface marker antigen CD45.

27. The method according to claim 21, wherein for the generation of human cells and/or tissues of the nervous system or neural stem cell progenitors a cell population is used containing at least 10 % of cells expressing the cell surface marker antigen CD133.

28. The method according to claim 21, comprising the steps of:

a) obtaining a mixed population of human cells comprising hematopoietic stem cell progenitors from peripheral blood and/or bone marrow and/or umbilical cord blood,

b) enrichment of the hematopoietic stem cell progenitors by selecting for those cells that are CD34 positive and/or CD117 positive and/or CD 133 positive,

and additionally to the enrichment step or instead of the enrichment step: depletion of those cells that are in a more differentiated state than the hematopoietic stem cell progenitors;

c) induction of the differentiation of the hematopoietic stem cell progenitors to cells and/or tissues of the nervous system or neural stem cell progenitors by placing the cell population comprising enriched hematopoietic stem cell progenitors in an appropriate cell-inducing environment.

29. The method according to claim 28, wherein the enrichment in step b) is performed by
contacting the mixed cell population with one or more reagents that bind to antigens selected from the group: CD34, CD117, CD133; and
selecting for cells that have bound the reagent, to produce a population enriched for hematopoietic stem cell progenitors.

30. The method according to claim 28, wherein in the depletion step cells are removed having cells surface antigens selected from the group: CD2, CD3, CD14, CD16, CD19, CD24, CD56, CD66b, Glycophorin A.

31. The method according to claim 28, wherein after the enrichment the cell populations are at least about 5 % of the cells present having the hematopoietic stem cell progenitors phenotype, preferably at least about 10 % of the cells present.

32. The method according to claim 28, wherein for the induction of the differentiation the cells are cultivated in a medium supplemented with growth factors which induce the differentiation of human hematopoietic stem cell progenitors into cells of the nervous system or neural stem cell progenitors.

33. The method according to claim 28, wherein for the induction of the differentiation the cells are cultivated in a medium supplemented with compounds selected from the group: EGF (epidermal growth factor), FGF-2 (fibroblast growth factor), FGF-1 with heparin, transferrin, selenite, progesterone, putrescine, insulin.

34. The method according to claim 28, wherein for the induction of the differentiation the cells are cultivated in a medium which is serum free.

35. The method according to claim 28, wherein for the induction of the differentiation the cells are cultivated in a medium which is supplemented with human serum.

36. The method according to claim 28, wherein the induction of the differentiation occurs in vivo.

37. A method for the generation of human cells and/or tissues of the nervous system or neural stem cell progenitors from human hematopoietic stem cell progenitors, comprising the steps of:

a) obtaining a mixed population of human cells comprising hematopoietic stem cell progenitors from peripheral blood and/or bone marrow and/or umbilical cord blood,

b) enrichment of the hematopoietic stem cell progenitors by selecting for those cells that are CD34 positive and/or CD117 positive and/or CD 133 positive,

and additionally to the enrichment step or instead of the enrichment step: depletion of those cells that are in a more differentiated state than the hematopoietic stem cell progenitors;

c) induction of the differentiation of the hematopoietic stem cell progenitors to cells and/or tissues of the nervous system or neural stem cell progenitors by placing the cell population comprising enriched hematopoietic stem cell progenitors in an appropriate cell-inducing environment.

38. A method for the generation of human cells and/or tissues of the nervous system or neural stem cell progenitors from human hematopoietic stem cell progenitor, comprising the steps of:

a) obtaining a mixed population of human cells comprising hematopoietic stem cell progenitors from peripheral blood and/or bone marrow and/or umbilical cord blood,

b) enrichment of the hematopoietic stem cell progenitors by selecting for those cells that are CD34 positive and/or CD117 positive and/or CD 133 positive by contacting the mixed cell population with one or more reagents that bind to antigens selected from the group: CD34, CD117, CD133, and selecting for cells that have bound the reagent,

to produce a population enriched for hematopoietic stem cell progenitors.

and additionally to the enrichment step or instead of the enrichment step: depletion of those cells that are in a more differentiated state than the hematopoietic stem cell progenitors, wherein cells are removed having cells surface antigens selected from the group: CD2, CD3, CD14, CD16, CD19, CD24, CD56, CD66b, Glycophorin A;

c) induction of the differentiation of the hematopoietic stem cell progenitors to cells and/or tissues of the nervous system or neural stem cell progenitors by placing the cell population comprising enriched hematopoietic stem cell progenitors in an appropriate cell-inducing environment.

39. A method for the generation of human cells and/or tissues of the nervous system or neural stem cell progenitors from human hematopoietic stem cell progenitor, comprising the steps of:

a) obtaining a mixed population of human cells comprising hematopoietic stem cell progenitors from peripheral blood and/or bone marrow and/or umbilical cord blood,

b) enrichment of the hematopoietic stem cell progenitors by selecting for those cells that are CD34 positive and/or CD117 positive and/or CD 133 positive by contacting the mixed cell population with one or more reagents that bind to antigens selected from the group: CD34, CD117, CD133, and selecting for cells that have bound the reagent, to produce a population enriched for hematopoietic stem cell progenitors.

and additionally to the enrichment step or instead of the enrichment step: depletion of those cells that are in a more differentiated state than the hematopoietic stem cell progenitors, wherein cells are

removed having cells surface antigens selected from the group: CD2, CD3, CD14, CD16, CD19, CD24, CD56, CD66b, Glycophorin A;

c) induction of the differentiation of the hematopoietic stem cell progenitors to cells and/or tissues of the nervous system or neural stem cell progenitors by placing the cell population comprising enriched hematopoietic stem cell progenitors in an appropriate cell-inducing environment, wherein for the induction of the differentiation the cells are cultivated in a medium supplemented with growth factors which induce the differentiation of human hematopoietic stem cell progenitors into cells of the nervous system or neural stem cell progenitors;

40. A method for the generation of human cells and/or tissues of the nervous system or neural stem cell progenitors from human hematopoietic stem cell progenitor, comprising the steps of:

a) obtaining a mixed population of human cells comprising hematopoietic stem cell progenitors from peripheral blood,

b) enrichment of the hematopoietic stem cell progenitors by selecting for those cells that are CD34 positive and/or CD117 positive and/or CD 133 positive by contacting the mixed cell population with one or more reagents that bind to antigens selected from the group: CD34, CD117, CD133, and selecting for cells that have bound the reagent, to produce a population enriched for hematopoietic stem cell progenitors;

and additionally to the enrichment step depletion of those cells that are in a more differentiated state than the hematopoietic stem cell progenitors, wherein cells are removed having cells surface antigens selected from the group: CD2, CD3, CD14, CD16, CD19, CD24, CD56, CD66b, Glycophorin A;

c) induction of the differentiation of the hematopoietic stem cell progenitors to cells and/or tissues of the nervous system or neural stem cell progenitors by placing the cell population comprising enriched hematopoietic stem cell progenitors in an appropriate cell-inducing environment, wherein for the induction of the differentiation the cells are cultivated in a medium supplemented with compounds selected from the group: EGF (epidermal growth factor), FGF-2 (fibroblast growth factor), FGF-1 with heparin, transferrin, selenite, progesterone, putrescine, insulin.

41. A composition of human cells comprising human hematopoietic stem cell progenitors, which are able to differentiate into cells and/or tissues of the nervous system or neural stem cell progenitors.

42. A composition of human cells comprising cells and/or tissues of the nervous system or neural stem cell progenitors, which were generated from human hematopoietic stem cell progenitors.

Fig. 1

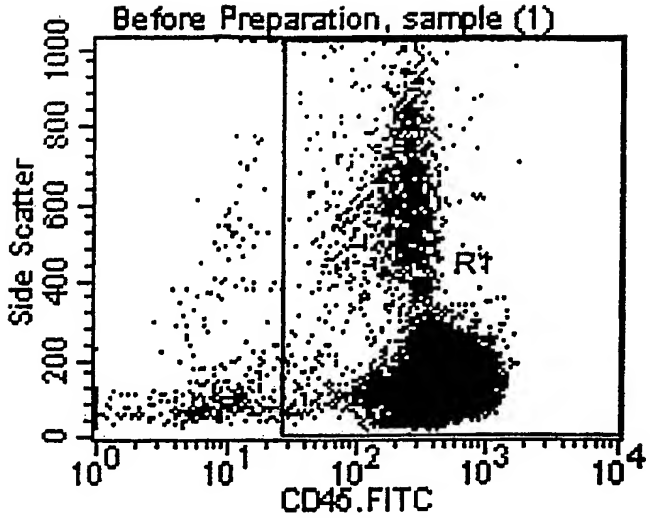


Fig. 2

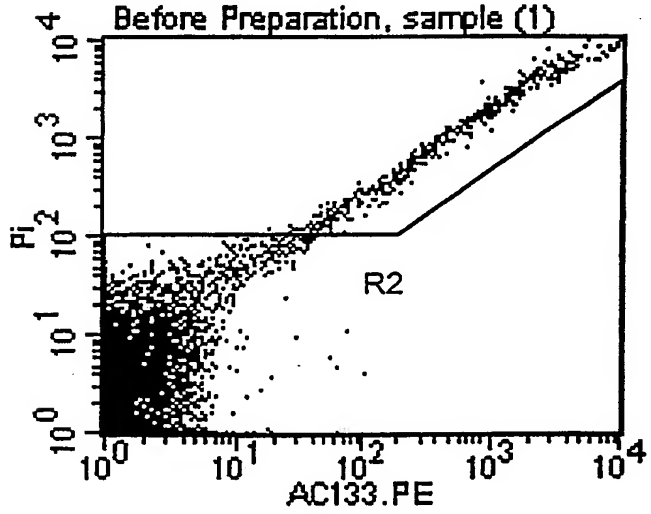


Fig. 3

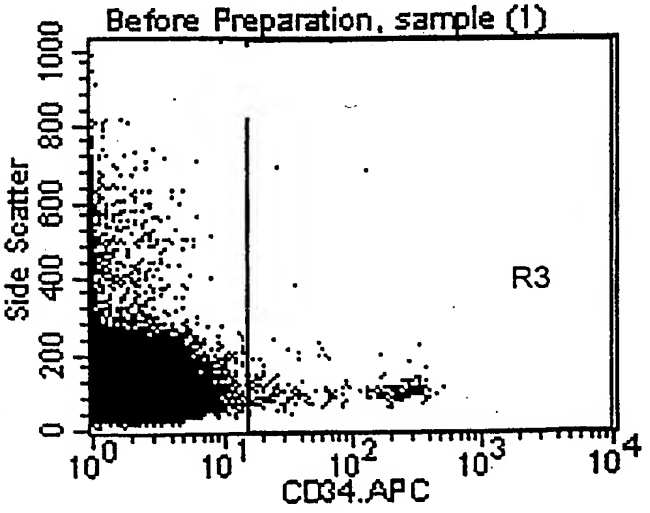


Fig. 4

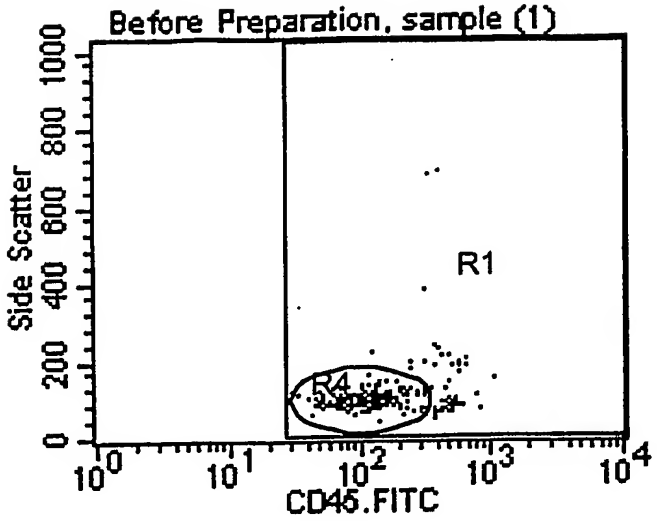


Fig. 5

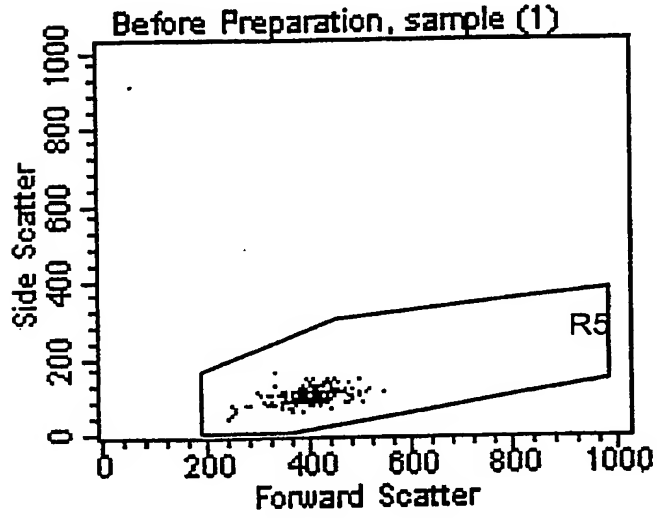


Fig. 6

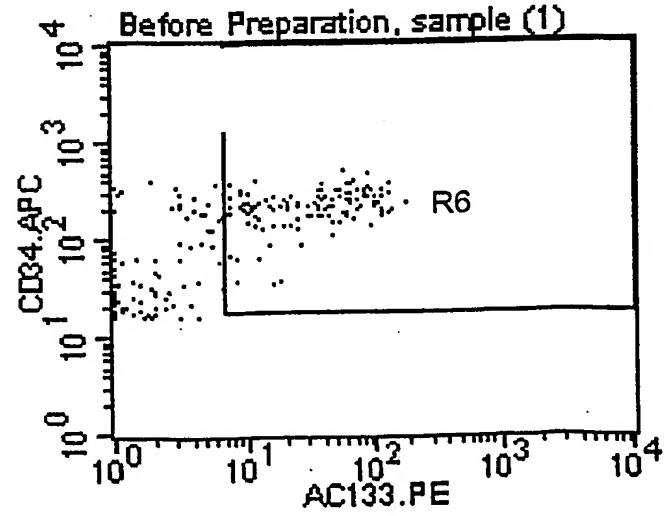


Fig. 7

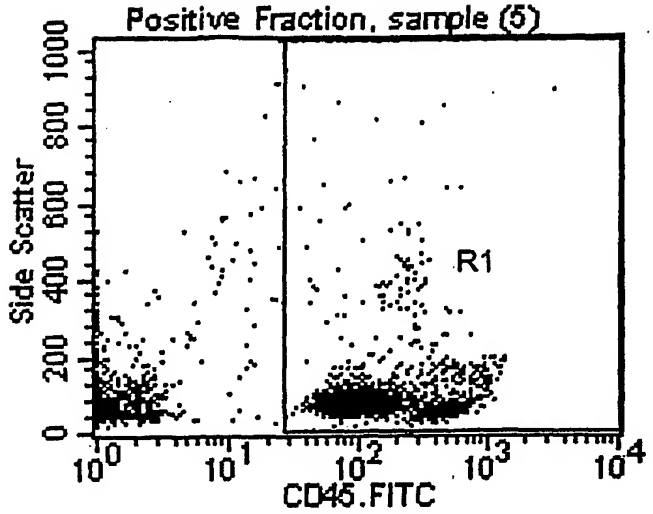


Fig. 8

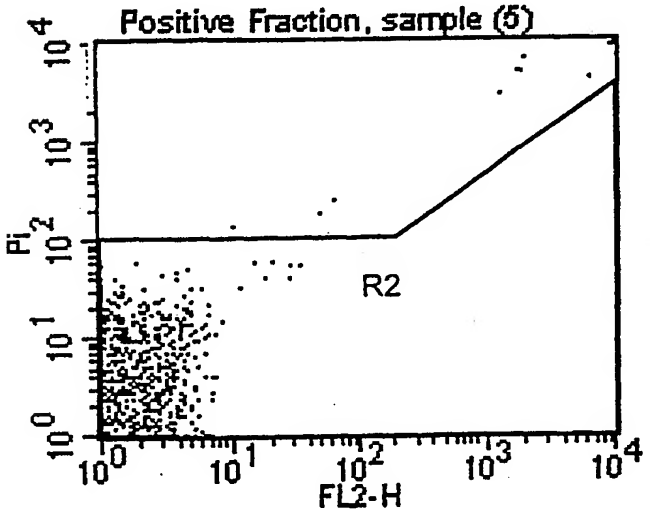


Fig. 9

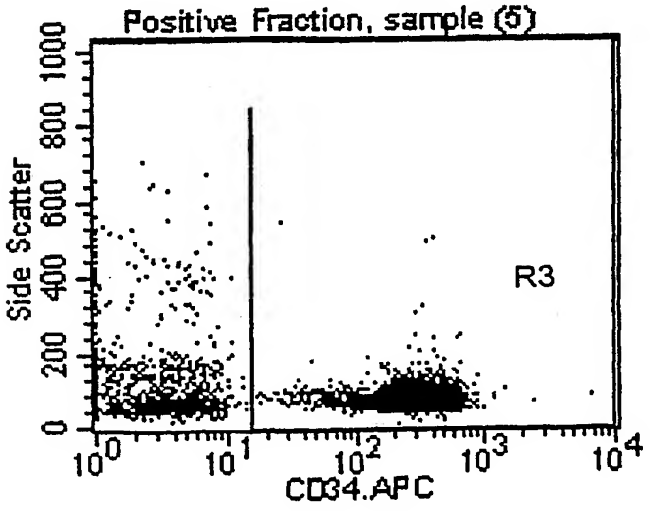


Fig. 10

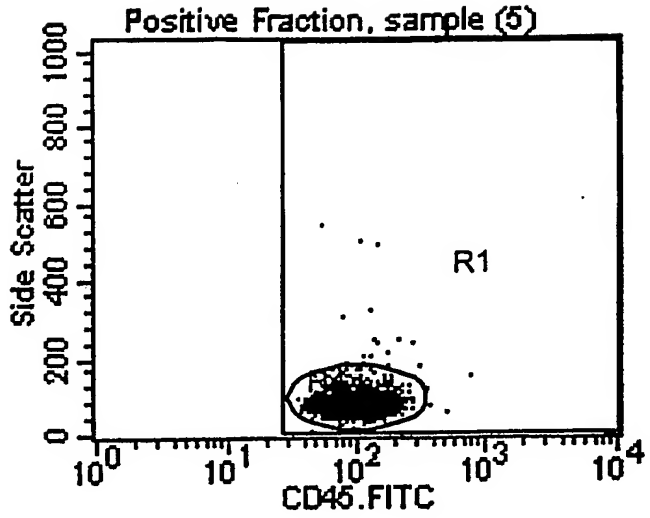
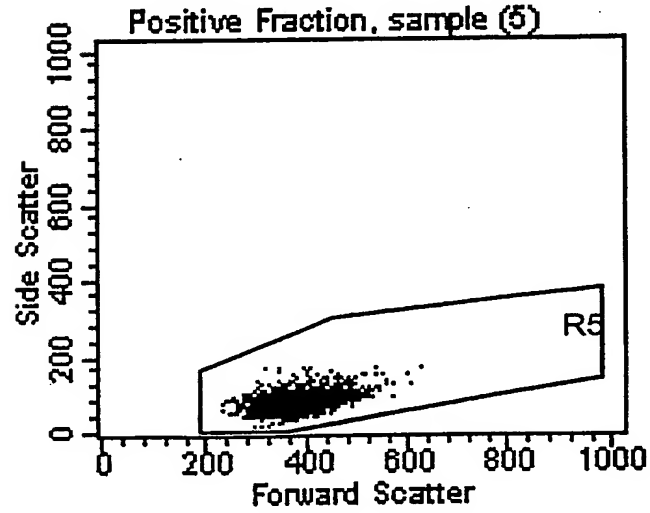


Fig. 11



INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/03097

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N5/06 A61K35/30

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FERRERO DARIO ET AL: "Mobilised peripheral blood progenitor cells give rise in vitro to cells expressing neuronal phenotype." BLOOD, vol. 98, no. 11 Part 2, 16 November 2001 (2001-11-16), page 123b XP001098866 43rd Annual Meeting of the American Society of Hematology, Part 2;Orlando, Florida, USA; December 07-11, 2001, November 16, 2001 ISSN: 0006-4971	1-12, 17-26
Y	abstract	13-15
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 October 2002	05.02.03

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Tudor, M

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LIOUTERMAN L ET AL: "Transplantation of human hematopoietic stem cells into rat brain: Analysis of cell survival and differentiation." SOCIETY FOR NEUROSCIENCE ABSTRACTS, vol. 27, no. 1, 2001, page 632 XP001098867 31st Annual Meeting of the Society for Neuroscience;San Diego, California, USA; November 10-15, 2001 ISSN: 0190-5295	1-11,16, 17,21-26
Y	abstract	12-15, 18-20

X	WO 01 75094 A (UNIV JEFFERSON) 11 October 2001 (2001-10-11)	1-11,16, 17,21-26
Y	the whole document	12-15, 18-20

X	BONILLA SONIA ET AL: "Haematopoietic progenitor cells from adult bone marrow differentiate into cells that express oligodendroglial antigens in the neonatal mouse brain." THE EUROPEAN JOURNAL OF NEUROSCIENCE. FRANCE FEB 2002, vol. 15, no. 3, February 2002 (2002-02), pages 575-582, XP002214223 ISSN: 0953-816X page 576, column 1, paragraph 2 page 578, column 2, paragraph 2 -page 581, column 1, line 3 page 581, column 1, line 4 - line 14 page 581, column 2, paragraph 4 -page 582, column 1, paragraph 1	1-11, 16-26

X	WO 95 05843 A (GALY ANNE ;DIGIUSTO DAVID (US); SYSTEMIX INC (US)) 2 March 1995 (1995-03-02) the whole document	21,22

Y	WO 95 12665 A (DIACRIN INC) 11 May 1995 (1995-05-11) page 15, line 1 - line 33	1-15, 17-26

Y	WO 01 68815 A (PERA MARTIN FREDERICK ;UNIV MONASH (AU); BEN HUR TAMIR (IL); REUBI) 20 September 2001 (2001-09-20) page 59, line 25 -page 60, line 29	1-15, 17-26

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PALMER T D ET AL: "Fibroblast growth factor-2 activates a latent neurogenic program in neural stem cells from diverse regions of the adult CNS." THE JOURNAL OF NEUROSCIENCE: THE OFFICIAL JOURNAL OF THE SOCIETY FOR NEUROSCIENCE. UNITED STATES 1 OCT 1999, vol. 19, no. 19, 1 October 1999 (1999-10-01), pages 8487-8497, XP001105835 ISSN: 1529-2401 page 8487 -page 8497 -----	12-15,20

INTERNATIONAL SEARCH REPORT

...tional application No.
PCT/EP 02/03097

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Due to the filing of two sets of claims this International Search Report has been carried out on claims 1-26 (see also reasonings for lack of unity of invention) of the first set of claims filed.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-26

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-26

   A method for generating cells or tissues of the human nervous system from haematopoietic stem cells derived from blood, bone marrow or umbilical cords; cells or tissue cultures derived from said method; medicaments containing the cells of said method; the use of the cells of said method for the manufacture of said medicaments.

2. Claims: 27-30

   A method for the maintenance and propagation of human haematopoietic stem cell progenitors.

INTERNATIONAL SEARCH REPORT

al Application No
PCT/EP 02/03097

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0175094 A	11-10-2001	WO 0175094 A1	11-10-2001
		US 2001038836 A1	08-11-2001
WO 9505843 A	02-03-1995	AU 685506 B2	22-01-1998
		AU 7676994 A	21-03-1995
		EP 0722331 A1	24-07-1996
		WO 9505843 A1	02-03-1995
		US 5681559 A	28-10-1997
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		WO 9512665 A1	11-05-1995
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		US 6432711 B1	13-08-2002
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		AU 4036101 A	24-09-2001
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